THE REDUCTION DIVISION IN THE MICROSPOROCYTES OF *AGAVE VIRGINICA*[1]

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(WITH PLATES XII–XIV)

This investigation, my fourth on the reduction karyokinesis, was undertaken to test the correctness of my former conclusions on a subject apparently beset with many difficulties, judging from the numerous contradictory reports of various observers. Having obtained a year's leave of absence from the university for travel and study, I prepared suitable material of *Agave virginica* L., which was found very favorable for my purpose. The stamens were collected and killed at various hours of the day during the last week in June and the first in July, 1907, a number of vigorous plants being in bloom on the campus of the Ohio State University. The killing fluid used was a weak chrom-acetic acid solution (0.3 per cent. chromic and 0.7 per cent. acetic in water). After imbedding in paraffin, the sections were cut 10–20 μ thick and stained on the slide. After experimenting with various stains and combinations, Heidenhain's iron-hematoxylin was found satisfactory, while Delafield's hematoxylin and the various safranin combinations gave very poor results. This was probably due to the readiness with which the cytoplasm took up and retained these stains. In the whole investigation great care was taken to have the sections correspond somewhat to the size of the nuclei, for sections too thick or too thin may frequently give misleading figures. The nuclei in the microsporocytes of Agave are comparatively small, 15–20 μ in diameter, so it was possible to obtain rather complete spirems and spindles with rather thin sections.

I am greatly indebted to Professor Dr. HANS SCHINZ, of the University of Zürich, where the major part of the investigation was carried on, for his kindly assistance and courtesy shown me during my stay in his laboratory.

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INVESTIGATION

Incipient stages of division.—The sporogenous tissue is differentiated very early in the young stamens and all vegetative divisions come to an end long before the earlier stages of the reduction division are apparent in the microsporocytes. There is therefore no danger in Agave of mistaking belated prophases of vegetative divisions for stages of reduction. The nuclei of the incipient sporocytes are quite small (*fig. 1*) and usually contain but one or two nucleoli and a rather coarse chromatin net, in which are prominent dark-staining granules. The cytoplasm is rather dense, with a spongy structure. As the sporocytes grow in size the nucleus enlarges considerably, and at various points in the enlarging net, masses of chromatin material appear (*fig. 2*). Studied in detail the net reveals single chromatin granules lying here and there in the linin meshes, and the clumps of chromatin also show definite granules (*fig. 2a*). These masses do not appear to be of a definite number, but approximate the reduction number of chromosomes. They continue to become more conspicuous as the early stages of division progress, until they have the appearance of true protochromosomes. The meshes of the net at the same time become larger, and the finer branches disappear, being probably withdrawn into the larger threads and masses, like the pseudopodia of a rhizopod. The linin network appears to be the active agent, the granules being merely carried apart or together as the linin is moving. I think that there is no question but that these masses are the "prochromosomes" of OVERTON and STRASBURGER. As stated, they are approximately of the same number as the reduction number of chromosomes. The evidence is strong that they represent pairs of individual chromosomes which are orienting themselves preparatory to the formation of the spirem. Since the massing and lengthening of these structures may not be synchronous, the apparent number need not necessarily agree with the reduction number. At this stage the definite pairing of the individual chromosomes must occur, and it may be that this is the only definite pairing during the whole ontogeny. The number of protochromosomes may also appear greater than the reduction number, for the accumulation may at first be taking place at two or more points of the paired chromosomes. It appears that the chromosomes, extended and spread out like a rhizopod or an

amoeba in the chromatin net, mass themselves together as definite individuals, probably in pairs; for thus alone are the later stages of reduction intelligible.

During these early stages the tapetum is in the very beginning of its development. It is therefore of value in helping to determine the exact sequence in the development of the sporocytes. And it is of course evident that if the successive stages cannot be determined with absolute certainty, the whole investigation is vitiated.

After the development of the chromatin masses, they seem to elongate, as is shown to some extent in *fig. 3*, and more perfectly in *fig. 4*. Finally they are stretched out into a very long and delicate continuous spirem, with rather uniformly distributed chromatin granules (*fig. 5*). The masses are probably all connected in series and thus elongate into a continuous delicate strand.

If the individuality of the chromosome is admitted, we may conceive the influence which causes paternal and maternal chromosomes to conjugate in pairs in reduction to be of much the same character as that which induces cells to develop as male and female gametes with subsequent union. This property may develop in the chromosomes only at the reduction stage, and if this were the case, the paternal and maternal units might be indifferent in regard to each other during all the vegetative divisions. The evidence on this point must come from normal crosses rather than from hybrids, where the two chromatins may have such a lack of affinity as not to conjugate at all. The independence of the maternal and paternal chromatin observed in the first few divisions of the fertilized egg is of a negative character. But if the individual chromatin masses are distinct in normal vegetative divisions, the pairing may nevertheless certainly take place at the formation of the protochromosomes. It may be well to insist here that the great extension, branching, and change of shape of the chromosome in the resting nucleus does not necessarily impair its individuality. We may reasonably consider the chromosomes to be individualized bodies, passing through a normal ontogeny and acting somewhat like the zoospores of a coenobic plant like Hydrodictyon or Pediastrum, which develop various properties at definite times to take them through their life cycle. They have a definite form, which recurs from generation to generation, but this is lost during the resting

stage, even as the cells of a myxomycete lose their individuality in the plasmodium.

First stages of synizesis.—As soon as the extended and delicate spirem is formed, the nuclei mostly appear in synizesis. There are all types of contraction. The chromatin may stretch across the center of the nuclear cavity (*figs. 6, 8*); it may be contracted at one side with the nucleolus (*fig. 7*); or it may be balled up at one side of the cavity with the nucleolus lying free (*fig. 10*). In some cases the mass is in the center, and often the nuclear membrane is injured by the irregular expansion of the nuclear cavity (*fig. 9*). The period of development during which synizesis occurs is comparatively long, the anther lengthening greatly in the meantime. The anthers of Agave thus make a most favorable object on which to determine definitely the stage when contraction must be looked for in the living material. A thorough study was therefore made of unstained as well as of stained sections, in order that I might become familiar with the appearance of the cells in unfixed anthers.

Study of living cells.—Having ascertained the stage when synizesis occurs in killed material, a study was made of living anthers during the last two weeks in June, 1908, at Columbus. The anthers were examined immediately after removal from the plant. In some cases cross-sections were cut, in others the stamens were cut into short pieces and the sporocyte tissue squeezed out and mounted in water. Both methods were satisfactory. In none of the numerous anthers studied during the two weeks was there the slightest evidence of synizesis. In the great majority of cases the nucleolus is in the center of the nuclear cavity; occasionally it is somewhat to one side; and very rarely near the nuclear wall, as is almost universal in the synizesis of the killed material. The nuclei look large, clear, and vesicular, with granules and flaky material (no doubt the chromatin) scattered throughout the cavity. The synizetic knot would certainly be visible were one present. In the killed material the synizetic mass shows nearly as distinctly in unstained as in stained material. The same undisturbed condition was found in all the cells in every possible stage in the period before chromosome formation. The fact that the nucleoli have a nearly uniform position near the center of the nuclear cavity, while in synizesis they are usually near or against the nuclear

wall or flattened out in the "sickle stage," is in itself sufficient proof that synizesis is an artifact. But as stated, the chromatin can also be faintly recognized in the living nuclei, and it should be still more evident if in a contracted ball, since the cavity in typical synizesis is entirely empty of threads, flakes, or granules. The granular material in the nucleus often radiates outward from the nucleolus, and sometimes it is prominently distributed over the surface of the nuclear membrane.

With salt solution and also with 95 per cent. alcohol, the cells contracted considerably and soon became indistinct, so that it was difficult to make out any details. The nuclei were displaced to some extent. The weaker chrom-acetic acid solution, used for the paraffin material, caused the whole mass of sporocytes to contract violently, but not much displacement of the nuclear contents was noticeable. This was probably because the cells were lying rather free and could contract readily from all sides, or the fluid may not have acted long enough. However, it is probable that the synizesis occurs rather suddenly.

An attempt was made to stain the fresh material, both before and after treatment with killing fluids; but this proved unsatisfactory, the stained material showing no more detail then the living cells.

A study of the living microsporocytes of *Agave virginica* indicates that synizesis, as seen in the usual paraffin sections, is an artifact. When the chromatin is comparatively free in the nuclear cavity and is expanding, we find the most decided synizesis. Meanwhile, as will appear further on, synizesis is not confined to this stage, but occurs to a greater or less extent until the chromosomes are fully developed. It is largely on account of the erroneous idea that synizesis occurs at but one stage of division that a number of inaccurate interpretations have been advanced, through which the whole subject of reduction has been confused.

Development of the chromatin loops.—The spirem begins to thicken while the chromatin granules are still in a single row (*figs. 11, 11a*). At this stage synizesis is still frequent, the spirem usually being crowded to one side, but occasionally lying entirely around the nuclear wall (*figs. 12, 13*). The spirem now becomes very distinct, so that it is often possible to trace out great lengths of the thread by focusing

properly. It begins to twist into loops and the chromatin granules now appear double (*figs. 14, 14a*). Although the spirem is much thicker at this stage than it was earlier, synizesis is occasionally present, the spirem filling one-half of the nuclear cavity, as shown in *fig. 15*. The double granules are at length prominent, although the spirem does not split (*figs. 16, 16a*). Finally the whole spirem is thrown into definite loops of various shapes and sizes. It is difficult to represent the perspective of these loops in a drawing. One can trace out the position and depth by focusing up and down, but in the camera projection they appear nearly in a plane (*figs. 17–20*). There is no question but that the spirem is continuous, since one can often follow the thread through more than half of the loops without losing the connection, and in uncut sections no free ends are present. In tangential sections or half-sections one can frequently also follow through three or four loops before coming to a free end (*fig. 19*). Practically also, it seems impossible that such twists and loops could be formed unless the spirem were continuous. In *fig. 18a* a number of twisted loops are shown. Some of the loops are produced by a single twist, which results in ring-shaped chromosomes (*fig. 20*). There are three of these ring-chromosomes in the nucleus and they are developed side by side. The three main types of loops are shown in *figs. 20a, 20b*, and *20c*. The loops are not formed, as in Lilium, with a central knot, but more openly. In this stage synizesis was also present in some of the material (*fig. 21*).

After the loops are developed, they are pressed and curved against the nuclear wall, the whole central part of the cavity becoming very hyaline. At the same time they break apart to form the individual reduction or bivalent chromosomes (*figs. 22, 23*). It was exceedingly difficult to determine the number of chromosomes on account of the irregular shapes of those bodies in some nuclei, as appears in *figs. 24, 25;* but it was finally determined that the number is twelve (*figs. 26–32, 37, 38*). In *fig. 26* only fragments of the twelve chromosomes are shown, a large part of the nucleus being cut away.

In cases where the number of chromosomes is said to vary somewhat, the greater or less number may not be of any significance, if the variation appears in vegetative division. Two or more chromosomes might become united through a failure of transverse segmentation,

but longitudinal division could proceed in the normal way and the identity of the chromosomes not be lost. But in reduction the number should be definite, if the karyokinesis is to furnish normal cells.

The nucleolus is still present when the chromosomes are fully developed, but often shows signs of fragmentation, as in the examples shown in *fig. 31a*. After the chromosomes are developed, the cytoplasm also shows a change in structure, having passed from a spongy or reticulate arrangement to a more or less radiate structure (*figs. 31, 32*).

Individuality of the chromosomes.—The chromosomes continue to become more indefinite in shape until they appear as irregular, dark-staining, apparently structureless masses, very unequal in size. The real character of the chromosomes can be studied to advantage only during the formative period, although the larger ones can be recognized even in the mother star. In the incipient chromosome loops individuality is very marked. As stated, there are three small ring chromosomes (*figs. 20, 20a, 29, 63*); four large long chromosomes, two of which are very prominently coiled and always side by side (*figs. 20b, 22, 27, 29, 30, 32, 63, 64*); and five smaller chromosomes of various shapes and sizes. Since these are bivalent chromosomes, it is evident that, on the theory of the conjugation of maternal and paternal chromosomes, the conjugating pairs must be quite similar in shape and activity. In the microsporocytes the bivalent chromosomes have an individual shape and size easily distinguishable, and the inference from this is evident, as also in the massing of the chromatin in the early prophase, that these bodies are individualized and retain their individuality from one division to another. Were the chromosomes not individualized, they could not preserve such definite forms and numbers from generation to generation. During its ontogeny, the chromosome passes through a series of forms, only to return, as any other organism, to a definite type at a definite stage. From the present study and the investigations of others, it is evident that the mechanics of chromosome reduction is simple, the usual spirem orienting itself into folds, twists, or simple loops, giving rise to all the various shapes, as rings, rods, coils, tetrads, and crosses. The actual form observed in any individual case may be a mere projection, and great care should be taken to ascertain the actual shape by observation from various points of view.

The spindle and late stages of synizesis.—The incept of the spindle is laid down immediately over the surface of the nuclear membrane while that structure is disappearing. At the same time, connecting lines, which appear prominently in heavy-stained sections, are present, forming a sort of network between the chromosomes (*figs. 37, 38*). The incipient spindle appears as a dense wall of material that was at first mistaken for the modified nuclear membrane, which, however, lies on the inside. This double layer about the nucleus, together with the connecting strands between the chromosomes, makes an ideal arrangement for abnormal contractions, and at this stage there is present a final prominent synizesis of the chromosomes, together with the dissolving nuclear membrane inclosing them. The chromosomes at this stage have not yet fused with the surrounding spindle. A few examples of this appearance are shown in *figs. 32–38*, all about in the same stage of division. Those which show the connecting fibers less distinctly are from the lighter-stained preparations. In fact, without a heavy stain, the connecting threads are barely visible. The contracted nuclei are seen in slides side by side with cells having a normal appearance. There is no doubt in the writer's mind that the phenomenon is an artifact.

The spindle.—The incipient spindle soon begins to show a fibrous character, the fibers at first being few and indistinct, and running more or less parallel toward opposite poles of the nucleus (*fig. 39*). In many cases two more or less pointed caps extend from opposite sides of the nucleus and become prominent before the longitudinal fibers are visible (*figs. 40–42*). The points sometimes show delicate asters, as in *figs. 41, 42*. The spindle fibers develop rapidly, and soon an oval slightly pointed structure is produced, in which the chromosomes and one or more nucleoli lie scattered about (*fig. 43*). The connecting fibers are also prominent, giving the spindle an irregular appearance. The spindle is bipolar from the beginning, originating and developing in the same way as in the vegetative divisions. FULLMER in 1899 showed that in Hemerocallis the spindle originates as a bipolar structure surrounded by a dark zone. This zone was nearly absent in Agave, but the difference may be due to staining. FULLMER showed, however, that the incipient spindle is entirely inside of the dark zone.

The spindle becomes narrower and more pointed and the connecting fibers, apparently contracting while the spindle is lengthening, gradually draw the chromosomes into a perfect circle in the equatorial plane (*figs. 44–60*). In *fig. 48* the spindle is distorted. This was probably produced by the unequal contraction of the cytoplasm. *Figs. 39, 43, 47, 57, 60, 61* make a series, showing how the chromosomes are drawn from their scattered positions into the symmetrical figure of the mother star. A large number of figures of this stage have been included in order to show all the ordinary types of developing spindles to be seen in Agave. In some, the connecting fibers are prominent; in others, especially as the chromosomes approach the equator, one sees only a dark-staining central mass. It is important to note that the spindle fibers appear thickest and densest in their central parts, even in very young spindles. Apparently the chromosomes are attached to the spindle fibers from the beginning. The crowding of the chromosomes against the nuclear wall, as shown in in *figs. 23, 39*, brings the chromosomes into a position where their fusion with the spindle fibers can be accomplished.

The mechanism for bringing the chromosomes from their scattered position into the symmetrical wreath of the mother star is comparatively simple, requiring only the shortening of the connecting fibers, combined with a pull from the spindle threads exerted from the poles. The action of the spindle as well as the attachments must be looked upon as being accomplished by a viscid substance, perhaps under the influence of attractive and repulsive forces. If the substance is contractile in the ordinary sense of the word, it must acquire this property after development.

Multipolar figures.—Multipolar figures were not numerous. This may have been because of the comparatively small size of the nuclei and the thickness of the sections. A special study was made of the multipolar figures found, and the conclusion was reached that they were all artifacts. The various types are shown in the series *figs. 64–73*. *Fig. 64* is a diagonal section, included to show the character and position of the chromosomes in the mother star. Both poles are cut away, one end more than the other. *Figs. 65, 66* are tangential sections representing small parts of the nucleus and spindle. The fibers are both spindle and connecting fibers and make an appear-

ance very much like the figures usually given to represent multipolar spindles. The writer believes that these connecting fibers have caused much trouble in the interpretation of spindle sections. *Fig. 67* might be taken for a tripolar spindle. The few projecting fibers were probably disturbed in the cutting. *Fig. 68* is a spindle broken and distorted by the knife. *Fig. 69* is another torn spindle, the fibers at one end being spread out by the knife. *Fig. 70* has the fibers of one pole cut diagonally. In *fig. 71* one pole is perfect, with a well-developed aster, while the other pole is cut away. In *figs. 72, 73* both poles have been cut off. Such figures are common, as is necessarily the case with cells in which the spindles lie in all directions.

The division of the bivalent chromosomes.—The chromosomes are arranged symmetrically in the mother star (*fig. 61*), with the closed end of the loop extending outward, at least in the long chromosomes (*fig. 61, a, b, c, d, e*). The spindle fibers are attached very near or at the free ends. In the following division the general appearance is entirely different. The larger chromosomes are V-shaped and are attached to the spindle fibers at the head of the V, the two free ends projecting outward (*fig. 62*). The individual character of the chromosomes may occasionally be seen from the polar view, even as late as the mother-star stage (*fig. 63*). The chromosomes are pulled apart very rapidly and are considerably scattered before they reach their new positions in the daughter stars (*figs. 74–77*). In some cells one can see large nucleoli in the cytoplasm along with micronucleoli (*figs. 57–59, 70, 75*).

The daughter chromosomes are arranged in a loose ring or plate, and then begin to contract, until they form a compact dark-staining mass (*figs. 78–82*). In the earlier stages of the daughter star, con ditions are again favorable for counting the chromosomes (*fig. 79*), and their smaller size is quite evident when compared with the bivalent chromosomes of the mother star. Delicate radiations are usually visible in all of these stages (*figs. 75–77, 81, 82*).

After the contraction stage of the incipient daughter nuclei, the chromatin begins to expand, the chromosomes putting out pseudopodia-like branches which become more extended until a coarse net is formed (*figs. 83–85*); but even in the oldest daughter nuclei distinguishable before the beginning of the following division, a con-

siderable part of each chromosome persists as an irregular compact mass (*fig. 85*). There is thus in these figures an indication that the individuality of the chromosome is preserved even in the chromatin network.

GENERAL CONSIDERATIONS ON REDUCTION

The important facts brought out in the present investigation confirm a number of conclusions put forward by the writer and others during the past ten years, most of which have been the subject of continual controversy. In a science like cytology so much depends on the manipulation of the material and the interpretation of the figures, that one need not be surprised at the diversity of views held in respect to all the more important cytological problems. In the present paper, by leaving out certain figures in the series, one can produce several of the "reduction processes" heretofore published.

The writer appears to have been the first to present a definite series of observations to show that the first division after pseudo-reduction is the real reduction division. A few previous reports had been published, which were, however, largely guesses or assertions, without definite evidence and sometimes even without drawings.

In 1897, the writer presented his views on the reduction division in the ovules of *Lilium philadelphicum*, advancing the definite con clusions that the spirem is continuous and contains a single row of chromatin granules which later undergo transverse fission; that the continuous spirem doubles up and twists into twelve loops, the reduction number, which then break apart at the inner ends opposite the heads of the loops to form the twelve chromosomes; that during metakinesis the two limbs of the chromosomes are pulled apart, finally breaking at the middle; and that, therefore, there is a transverse division in the first reduction karyokinesis, or a true qualitative division of the chromatin. In that paper *figs. 1, 2, 2a, 4, 4a, 8, 8b, 11, 11b, 12, 15, 21a, 22, 23, 23b, 34, 35* formed a series for which only one interpretation was possible. Only by leaving out *fig. 4* could another interpretation be given, in which case the double spirem appearing later might be considered as conjugating instead of dividing.

In 1901 practically the same results were obtained for Erythronium, and in 1906 for the microsporocytes of *Lilium tigrinum*.

PAULMIER in 1899 showed that in the spermatogenesis of *Anasa tristis* the first division is the reduction division, and more recently MONTGOMERY, in a series of important investigations, has come to the same conclusion. GRIGGS found a reducing division in Ascaris, and observed that the chromosomes are not entirely separated until they are drawn into the equatorial plane.

MOTTIER after a long-continued study of Podophyllum, *Lilium Martagon*, and other plants, has come to conclusions for the most part similar to the writer's, although for many years he held opposite views.

GATES, in a recent article, finds that in the reduction nucleus of the microsporocytes of *Oenothera rubrinervis* the spirem segments transversely into the $2x$ or sporophyte number, and that the members of a pair are thus at first arranged end to end on a single thread. Later the univalent chromosomes are separated, usually in pairs.

It is needless to review the extensive recent literature of reduction, for in many cases the results appear radically different from those presented in this report, and in examining the drawings and conclusions based on them there seems little possibility of harmonizing or explaining the differences.

Finally, it may be said that if any individuality whatever is ascribed to the chromosomes, it becomes evident that they should be arranged end to end to form the spirem, since this is the method in somatic divisions. It is not probable that the cell would develop a fundamentally new method of division in reduction, but rather that such slight changes would be developed in the process as would suffice to bring about the separation of the two sets of chromosomes. The process described in this paper appears to the writer to be the only possible explanation of the figures. As has been stated, however, by making suitable selections, one could represent almost any of the various reduction karyokineses that have been described.

SUMMARY

1. The resting nucleus in the microsporocytes of *Agave virginica* contains a linin network in which small chromatin granules are held, either separate or in lumps.

2. At the beginning of division, the chromatin granules are massed

together through the massing of the linin into a number of lumps corresponding approximately to the reduced number of chromosomes. These masses probably represent bivalent protochromosomes.

3. The masses are all united and elongate greatly until a very delicate, continuous spirem is produced, holding a single row of chromatin granules.

4. After the delicate spirem stage the nuclei in killed material are usually in synizesis. There is no union of two spirems in synizesis.

5. In living material no synizesis is evident, and the nucleoli are not crowded against the nuclear wall, but usually have a central position in the nuclear cavity. Synizesis at this as well as at later stages is an artifact.

6. The spirem shortens and thickens while the chromatin granules undergo transverse division. It finally orients itself into twelve loops of different shapes and sizes.

7. The loops are pressed close to the nuclear membrane, forming a rather definite wreathlike circle, and do not radiate from a closely entangled central mass as in Lilium.

8. The twelve loops break apart, forming the twelve chromosomes —four very large, long, twisted chromosomes; three ring-shaped chromosomes; and five smaller, irregular, more or less bean-shaped chromosomes.

9. The chromosomes are united by connecting fibers, which apparently contract and draw the scattered chromosomes into the equatorial plane while the spindle is elongating.

10. One or two nucleoli are usually present, which are still normal in appearance after the spindle is far advanced in development. The nucleoli are sometimes thrown out bodily into the cytoplasm.

11. The spindle originates as a more or less fibrous layer over the surface of the nuclear membrane before that body dissolves, and at this stage decided synizesis of the chromatin is often present.

12. The spindle is bipolar from the first, with no accessory smaller poles, the poles appearing at first as two, more or less pointed, dome-shaped caps, much the same as in vegetative karyokinesis.

13. The spindle fibers are usually most prominent and thickest in the middle, even in the early stages. There are commonly definite asters at the poles.

14. The multipolar spindles observed are explained as artifacts, mostly produced by cutting.

15. The chromosomes divide transversely during metakinesis.

16. In the daughter nuclei, irregular masses of chromatin persist into the resting condition. These masses represent parts of the twelve daughter chromosomes.

17. In the second division the chromosomes divide longitudinally.

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EXPLANATION OF PLATES XII–XIV

The plates are reduced five-eighths in reproduction. *Figs. 2a, 11a, 14a, 16a, 17a, 18a, 20a, 20b, 20c* were drawn with a compensating ocular 18 and oil immersion objective $\frac{1}{12}$; all the rest with a compensating ocular 12 and oil immersion objective $\frac{1}{12}$, the latter combination having a magnification on the table of 2250.

PLATE XII

FIG. 1.—Microsporocyte showing resting chromatin network.

FIG. 2.—Microsporocyte at a later stage showing masses of chromatin granules in the net.

FIG. 2*a*.—A small portion of the chromatin net showing the linin and massing of the granules.

FIG. 3.—A nucleus with prominent massing of the chromatin into rather definite bodies, protochromosomes.

FIG. 4.—Later stage; the chromatin masses stretching out into a definite spirem.

FIG. 5.—The delicate slender spirem complete.

FIG. 6.—Somewhat older than *fig. 5;* synizesis of the spirem in the middle of the nuclear cavity.

FIGS. 7–10.—Other types of synizesis of the same stage as *fig. 6;* in *fig. 9* the nuclear cavity is expanded.

FIG. 11.—Nucleus with spirem becoming thicker

FIG. 11*a*.—Single chromatin threads from *fig. 11*, showing the light-staining linin with a single row of chromatin granules

FIGS. 12, 13.—Types of synizesis in a later stage than those of *figs. 6–10.*

FIG. 14.—Continuous spirem beginning to fall into loops, showing chromatin granules enlarged and partly double.

FIG. 14*a*.—A short piece of the spirem from *fig. 14*, showing the double nature of the chromatin granules.

FIG. 15.—Same stage as *fig. 14*, showing one-sided synizesis of the continuous spirem.

FIG. 16.—Beginning of the looped spirem, showing further thickening and doubling of the chromatin granules.

FIG. 16*a*.—Pieces of the spirem from *fig. 16*, showing double rows of chromatin granules and distinct linin.

FIG. 17.—Continuous spirem, much thickened and thrown into twisted loops.

FIG. 17*a*.—Pieces of the spirem showing the method of looping and twisting.

FIG. 18.—Microsporocyte somewhat later than *fig. 17*, showing further thickening of the thread and development of the chromatin loops.

FIG. 18*a*.—A number of chromatin loops before the breaking of the spirem.

FIG. 19.—Section of microsporocyte in which several loops can be followed out; the section represents nearly half of the spirem.

FIG. 20.—Beginning of the broken skein stage; the chromosomes beginning to break apart; three ring-chromosomes still connected.

FIGS. 20*a*, 20*b*, 20*c*.—Three complete chromosomes just after the breaking of the spirem.

FIG. 21.—Synizesis in microsporocyte at time of separation of chromosomes.

FIG. 22.—Nucleus with chromosomes completely separated; nuclear membrane still present.

FIG. 23.—Somewhat later stage; chromosomes all crowded against the nuclear wall with a clear cavity in the center.

FIGS. 24, 25.—Nuclei showing indefinite chromosomes.

FIG. 26.—Section of nucleus showing parts of twelve chromosomes.

FIGS. 27, 28.—Nuclei showing twelve chromosomes of diverse shapes and sizes.

FIG. 29.—Section of nucleus showing the three ring-chromosomes.

PLATE XIII

FIGS. 30, 31.—Nuclei with twelve chromosomes, showing the beginning of the appearance of delicate connecting fibers.

FIG. 31*a*.—Fragmenting nucleoli taken from same stages as *fig. 31*.

FIGS. 32–36.—Microsporocytes showing synizesis after the formation of the chromosomes; also connecting fibers between the chromosomes.

FIG. 37.—Nucleus contracted away from the incipient spindle; prominent connecting fibers between the chromosomes.

FIG. 38.—The same, but with less synizesis of the nucleus.

FIG. 39.—Nucleus showing distinctly the incipient spindle.

FIG. 40.—Incept of spindle showing as two caps on opposite sides of the nucleus.

FIG. 41.—Nucleus showing incipient spindle.

FIG. 42.—Nucleus with incept of spindle and aster at one pole.

FIG. 43.—Nucleus showing young spindle and connecting fibers between the chromosomes.

FIGS. 44–46.—Further successive stages in the development of the spindle.

FIG. 47.—Chromosomes, connected by fibers, being drawn into the equatorial plane; spindle with aster showing at one pole.

FIG. 48.—Distorted spindle.

FIG. 49.—Spindle showing the two poles.

FIGS. 50–59.—Successive stages in the development of the spindle and the shifting of the chromosomes into the equatorial plane. *Figs. 57–59* on *plate XIV*.

PLATE XIV

FIG. 60.—Spindle, showing asters and centrosomes; the chromosomes nearly in the equatorial plane.

FIG. 61.—Mother star with aster at the poles.

FIGS. 61*a*, 61*b*, 61*c*, 61*d*, 61*e*.—Chromosomes on the spindle fibers, showing that the closed loop extends outward.

FIG. 62.—A chromosome from the mother star of the second division with the free ends of the V projecting outward.

FIG. 63.—Polar view of chromosomes, showing the three ring-chromosomes; four long chromosomes, two of which lie side by side and are very large; and five smaller chromosomes of various shapes and sizes.

FIG. 64.—Diagonal section of mother star, showing the twelve chromosomes, three of which occupy a central position; also the four long chromosomes.

FIG. 65.—A tangential section of a young spindle, showing spindle and connecting fibers.

FIG. 66.—Tangential section of a spindle, making a multipolar figure.

FIG. 67.—Section showing tripolar figure.

FIG. 68.—Section showing spindle torn by the knife.

FIG. 69.—Torn section, showing spindle fibers cut and spread apart by the knife.

FIG. 70.—Spindle with poles cut away showing two large nucleoli in the cytoplasm outside of the spindle.

FIG. 71.—Spindle, showing pole and aster at one end, the other pole being cut away.

FIG. 72.—Spindle with both poles cut off.

FIG. 73.—Another spindle with both poles cut.

FIG. 74.—Spindle showing first stage of metakinesis, the two large chromosomes being to one side.

FIG. 75.—Metakinesis stage.

FIG. 76.—First stage of daughter star, showing the separated chromosomes.

FIG. 77.—Daughter star stage.

FIG. 78.—Late daughter stars.

FIG. 79.—Daughter star, showing the twelve small chromosomes.

FIG. 80.—Loose daughter skein stage, showing the beginning of contraction of the chromosomes.

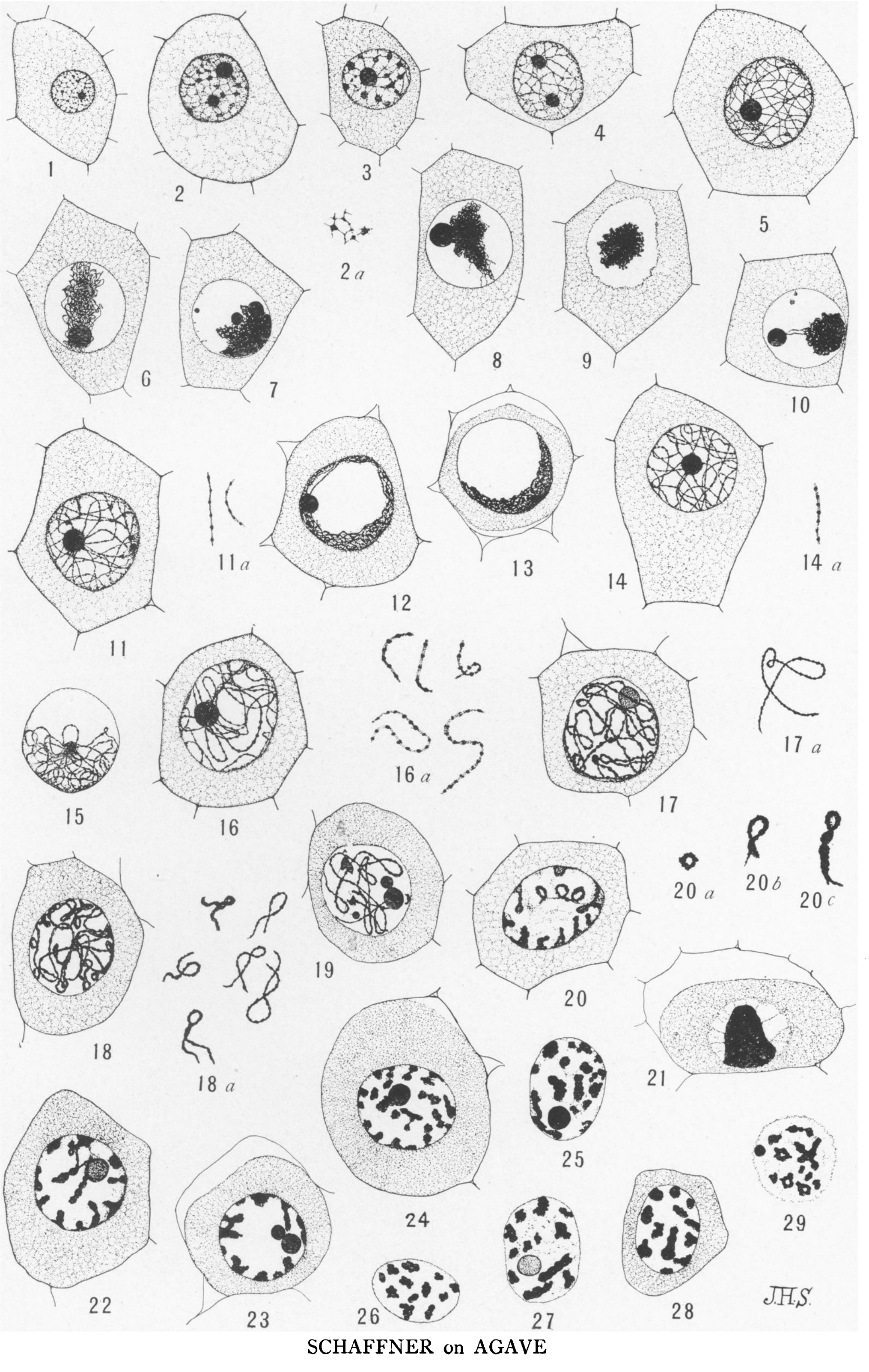
FIG. 81.—Daughter skein, showing the close massing of the chromatin.

FIG. 82.—Daughter skein, showing close massing of the chromosomes below the pole.

FIG. 83.—Beginning of formation of daughter net, showing the irregular daughter chromosomes.

FIG. 84.—Further development of the daughter net.

FIG. 85.—Resting stage of daughter nucleus, the chromosomes being still evident as irregular masses.



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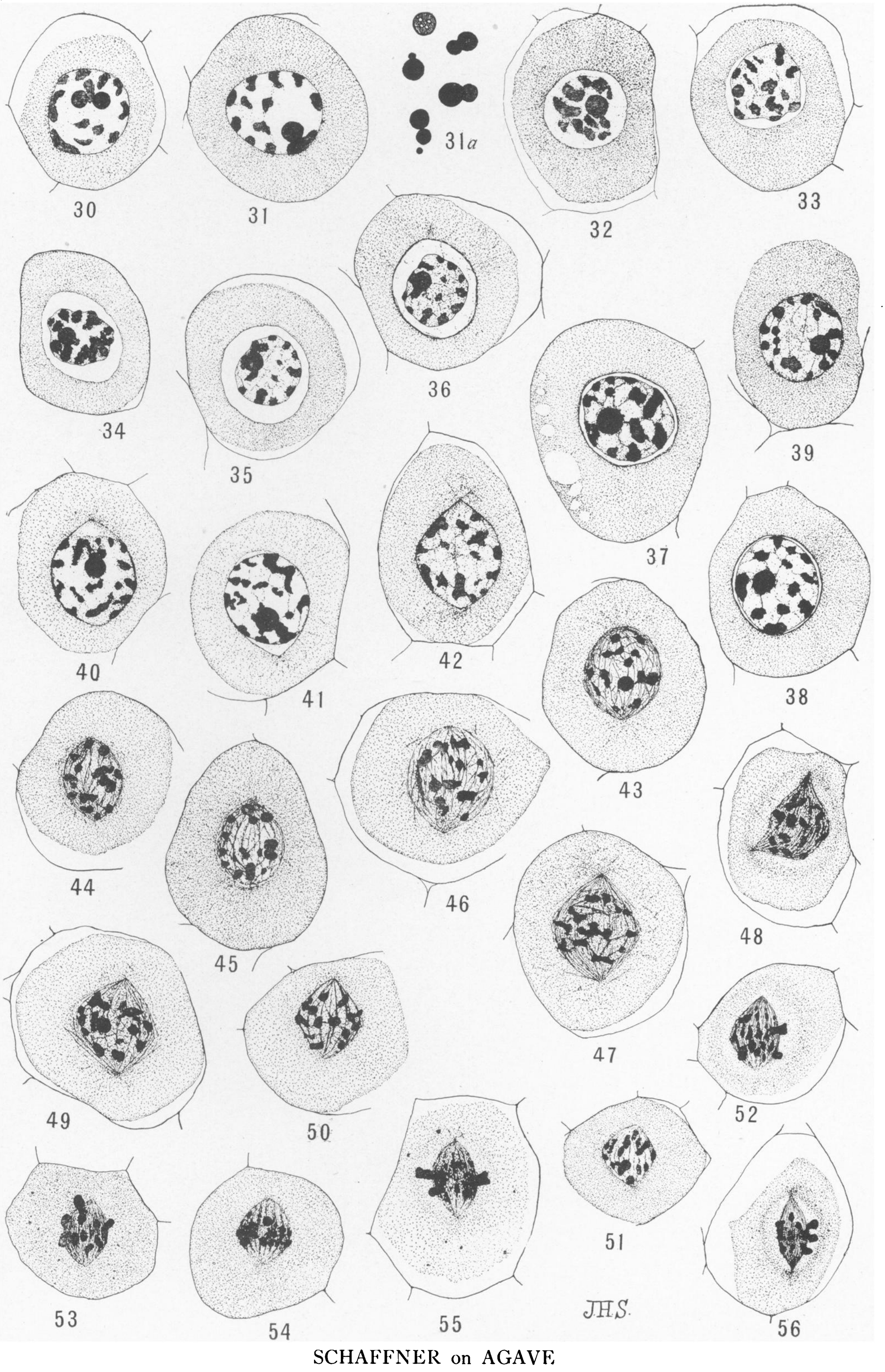

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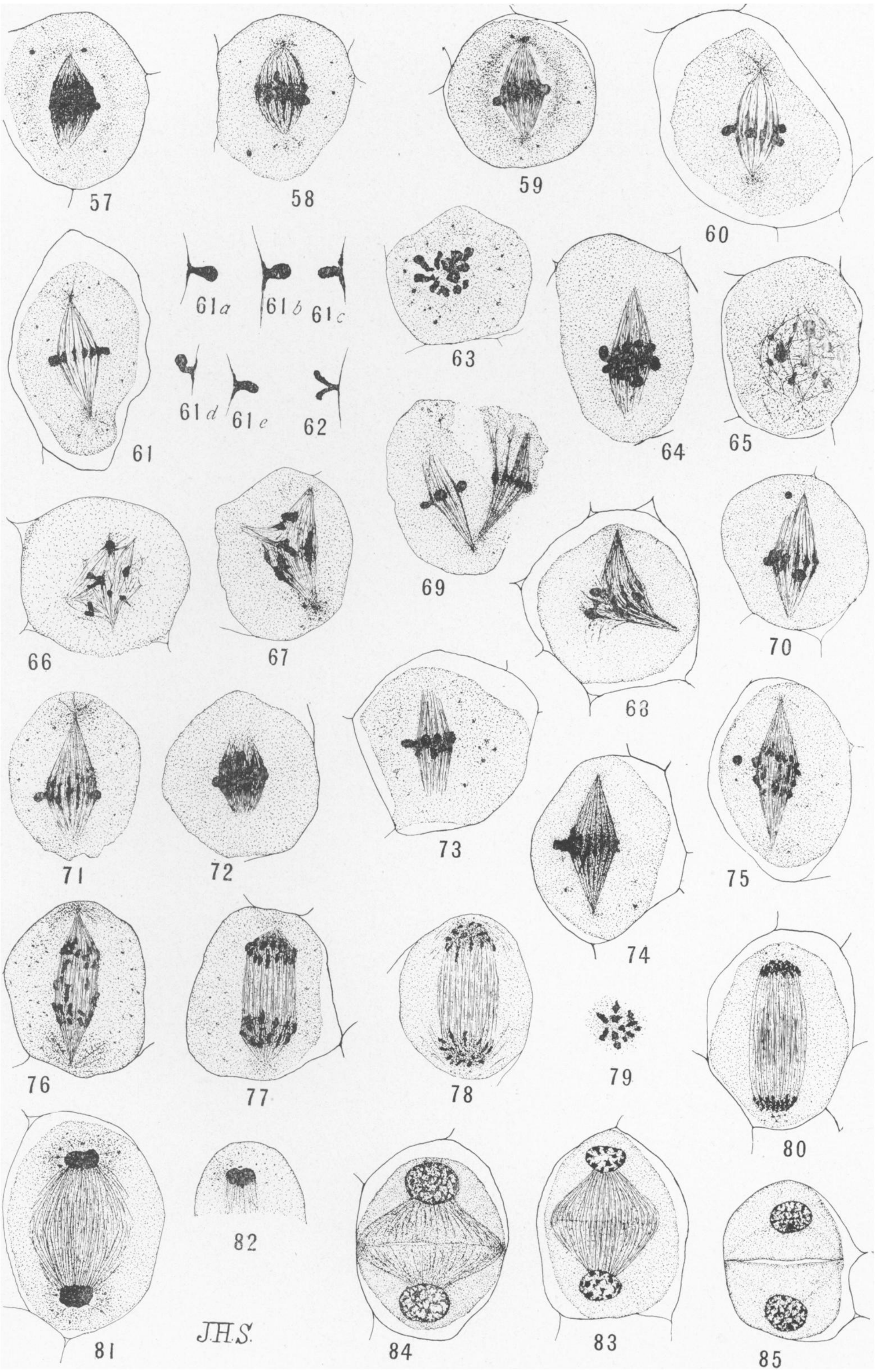

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